Rec'd PCT/PTO 27 MAR 1997

FORM PTO-1390 (REV 10-96) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 07856-0007

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5):

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP95/03757	22 Sept. 1995 (22.09.95)	28 Sept. 1994 (28.09.94)

TITLE OF INVENTION: PEPTIDES FROM THE hPTH SEQUENCE

APPLICANT(S) FOR DO/EO/US: ADERMANN, Knut; HOCK, Dieter; MAGERLEIN, Markus

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND or SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US)
6. [X] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [X] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [X] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information: Verified Statement for Small-Entity Status

EXPRESS MAIL LABEL NO. EM457482646US

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
	PCT/EP95/03757	07856-0007

17. [X] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):	CALCULATIONS PTO USE ONLY
Search Report has been prepared by the EPO or JPO $910.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482) $700.00	
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).. $770.00	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $1040.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $96.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 910.00
Surcharge of **$130.00** for furnishing the oath or declaration later than [20] [30] months from the earliest claimed priority date (37 CFR 1.492(e)).	$ --

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	6 -20=	0	X $22.00	$ --
Independent claims	2 -3 =		X $80.00	$ --
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+$260.00	$ --
TOTAL OF ABOVE CALCULATIONS =				$ 910.00
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				$ 455.00
SUBTOTAL =				$ 455.00
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$ --
TOTAL NATIONAL FEE =				$ 455.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$ 40.00
TOTAL FEES ENCLOSED =				$ 495.00
			Amount to be: refunded	$
			charged	$

a. [XX] A check in the amount of $ 495.00 to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 10-1215. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Roger T. Frost
Jones & Askew, LLP
191 Peachtree St., #3700
Atlanta, GA 30303

SIGNATURE:

Roger T. Frost
NAME

22,176
REGISTRATION NUMBER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

KNUT ADERMANN, ET AL.

App. No. _____
(National Phase of PCT/EP95/03757 filed September 22, 1995)

Filed: _____

For: **PEPTIDES FROM THE hPTH SEQUENCE**

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to calculating the total amount of filing fee required to enter the national phase in the application identified above, please enter the following amendments in that application.

In the Claims

Cancel Claims 1 and 2, without prejudice.

Please enter the following new Claim 7:

7. The peptides from hPTH (1-37) having the sequence

hPTH 1-10

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-Asn^{10}-OH (1)

EXPRESS MAIL LABEL NO. EM457482646US

Dated: March 27, 1997

hPTH 1-9

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-OH (2)

hPTH 1-8

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-OH (3)

hPTH 1-7

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-OH (4)

hPTH 1-6

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-OH (5)

hPTH 1-5

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-OH (6)

hPTH 9-18

NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (7)

hPTH 10-18

NH_2-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (8)

hPTH 11-18

NH_2-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (9)

hPTH 12-18

NH_2-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (10)

hPTH 13-18

NH_2-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (11)

hPTH 14-18

NH_2-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (12)

hPTH 9-17

NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-OH (13)

hPTH 9-16

NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-OH (14)

hPTH 9-15

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-OH (15)

hPTH 9-14

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-OH (16)

hPTH 9-13

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-OH (17)

hPTH 24-37

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-
Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (18)

hPTH 25-37

NH_2-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-
Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (19)

hPTH 26-37

NH_2-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-
Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (20)

hPTH 27-37

NH_2-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-
Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (21)

hPTH 28-37

NH_2-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-
Leu^{37}-OH (22)

hPTH 29-37

NH_2-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (23)

hPTH 30-37

NH_2-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (24)

hPTH 31-37

NH_2-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (25)

hPTH 32-37

NH_2-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (26)

hPTH 33-37

NH_2-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (27)

hPTH 24-36

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-OH (28)

hPTH 24-35

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-OH (29)

hPTH 24-34

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-OH (30)

hPTH 24-33

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-OH (31)

hPTH 24-32

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-OH (32)

hPTH 24-31

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-OH (33)

hPTH 24-29

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-OH (34)

hPTH 24-28

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-OH (35)

Claim 3, line 1, cancel "claim 1 and/or 2" and substitute --Claim 7--.

Claim 4, lines 3 and 4, cancel "at least one of claims 1-3" and substitute --Claim 7--.

Claim 4, line 7, cancel "at least one of claims 1-3" and substitute --Claim 7--.

Claim 5, line 3, cancel "at least one of claims 1-3" and substitute --Claim 7--.

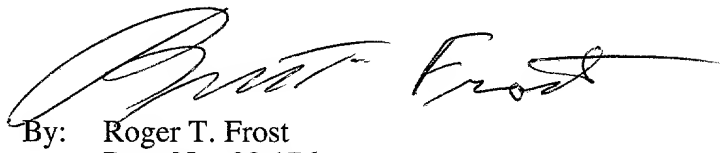
Claim 6, lines 1 and 2, cancel "at least one of claims 1-3" and substitute --Claim 7--.

REMARKS

These amendments conform the claims of this application to the claims appearing in the International Preliminary Examination Report. Moreover, the present amendments remove all multiple dependencies from the claims, thereby reducing the total amount of filing fee required for this application.

The applicant requests entry of this amendment and awaits examination in due course.

Respectfully submitted,



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Peptides from the hPTH(1-37) Sequence

The present invention relates to peptides from the sequence of hPTH(1-37), and the use of said peptides in the preparation of an agent for diagnosing biologically active hPTH.

Human parathyroid hormone (hPTH), a linear polypeptide having 84 amino acids, plays an important role in the regulation of the calcium metabolism. The metabolism of this hormone gives rise to a large number of C-terminal fragments, the biological functions of which have not yet been elucidated. The hPTH(1-37) has been established as a circulating N-terminal fragment (EP-A 0 349 545). This fragment has the full biological activity of the entire hormone. However, upon loss of the first amino acid, serine, the activity significantly decreases and is lost completely without the first two amino acids, serine and valine.

Serum levels in the range of 10^{-12} mol/l are measured for the intact hormone hPTH(1-84) and for the N-terminal fragment. Immunological measuring procedures are employed to determine such low concentrations. Here, the most valid results are provided by measuring procedures according to the double antibody or sandwich principle (e.g., the two-site radioimmunometric assay, IRMA, or the sandwich enzyme-linked immuno sorbent assay, Sandwich ELISA). For hPTH(1-84), such assays are commercially available. For the measurement of hPTH(1-34), an assay according to the double antibody principle is not known.

Here, two antibodies are required. In order to avoid mutual steric hindrance, they must be capable of recognizing antigen epitopes located at a sufficient distance from each other. When immunizing using the intact antigen, a heteroge-

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neous mixture of various antibodies is obtained, which first must be subjected to an expensive purification in order to conduct a sandwich assay. According to theoretical calculations by B.A. Jameson and H. Wolf, The Antigenic Index: A Novel Algorithm for Predicting Antigenic Determinants, CABIOS 4, p. 181-186, 1988; it has been possible so far to detect a preferred sequence having immunogenic activity in the region of the amino acids 7-14 at the N-terminus. Immunization with N-terminal fragments according to established methods predominantly results in antibodies which, as has been described for hPTH(1-34) (J. Tampe, P. Brozio, H.E. Manneck, A. Mißbichler, E. Blind, K.B. Millers, H. Schmidt-Gayk, and F.P. Armbruster, Characterisation of Antibodies Against Human N-Terminal Parathyroid Hormone by Epitope Mapping; J. Immunoassay 13, p. 1-13, 1992), bind in the region of these amino acids. However, these antibodies are not capable of discriminating between biologically active and biologically inactive PTH(1-84) or fragments thereof lacking the first two amino acids serine and valine.

The technical problem which this invention is based upon is to provide peptides by means of which it is possible to eliminate the above-mentioned drawbacks in the diagnosis of biologically active hPTH.

Surprisingly, the technical problem described above is solved by means of the following peptides from the sequence of hPTH(1-37):

hPTH 1-10
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-Asn^{10}-OH (1)

hPTH 1-9
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-OH (2)

hPTH 1-8
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-OH (3)

hPTH 1-7
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-OH (4)

hPTH 1-6
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-OH (5)

hPTH 1-5
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-OH (6)

hPTH 9-18
NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (7)

hPTH 10-18
NH_2-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (8)

hPTH 11-18
NH_2-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (9)

hPTH 12-18
NH_2-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (10)

hPTH 13-18
NH_2-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (11)

hPTH 14-18
NH_2-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (12)

hPTH 9-17
NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-OH (13)

hPTH 9-16
NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-OH (14)

hPTH 9-15
NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-OH (15)

hPTH 9-14
NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-OH (16)

hPTH 9-13
NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-OH (17)

hPTH 24-37
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-
Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (18)

hPTH 25-37
NH_2-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-
Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (19)

hPTH 26-37
NH_2-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-
Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (20)

hPTH 27-37
NH_2-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-
Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (21)

hPTH 28-37
NH_2-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (22)

hPTH 29-37
NH_2-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (23)

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hPTH 30-37

NH_2-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (24)

hPTH 31-37

NH_2-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (25)

hPTH 32-37

NH_2-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (26)

hPTH 33-37

NH_2-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (27)

hPTH 24-36

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-OH (28)

hPTH 24-35

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-OH (29)

hPTH 24-34

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-OH (30)

hPTH 24-33

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-OH (31)

hPTH 24-32

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-OH (32)

hPTH 24-31

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-OH (33)

hPTH 24-29

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-OH (34)

hPTH 24-28

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-OH (35)

The indicated sequences represent essential features of the secondary structure in their primary structure, as can be demonstrated by supporting NMR data. One precondition to this end was a determination of the PTH(1-37) secondary structure in physiological solution.

The above-mentioned regions of conspicuous structure have good immunogenic activity. Antibodies are formed, binding to the first amino acids of the N-terminus. Deficiency of only two amino acids gives rise to a substantial loss in affinity. Because these amino acids are indispensable for the biological activity to arise, it is possible by using the peptides of the invention to obtain antibodies recognizing only hPTH and fragments thereof which are biologically active.

Furthermore, antibodies can be produced which detect the mid-region 9-15, as well as antibodies giving C-terminal binding in the region of the amino acids 30-37. According to the invention, it is therefore possible to produce antibodies against hPTH(1-37) regions which, according to theoretical calculations, do not exhibit immunogenic activity within the entire molecule. In addition, these regions are separated from each other by such a far distance that no steric hindrance is present which would prevent simultaneous binding of two antibodies.

In preferred embodiments, the peptides may be modified at the N-terminal end, in the side-chain and/or at the C-terminal end, namely, taking the form of acetylation, amidation, phosphorylation and/or glycosylation products.

Eventually, the peptides of the invention may also be bound to carrier proteins such as hemocyanin, thyroglobulin, bovine serum albumin, ovalbumin, or mouse serum albumin etc.. Binding to the carrier proteins is preferably effected using carbodiimide or formaldehyde.

The peptides of the invention may be used in the preparation of a diagnostic agent. The diagnostic agent of the invention can be obtained using the per se known immunization of animals with at least one of the peptides according to the invention. Following immunization, an immunoglobulin fraction can be isolated from the immunized animals, which contains antibody fractions having an antibody titer against at least one of the peptides of the invention. The invention is also directed to the antibodies thus obtained. In addition to the complete antibodies consisting of F_{ab} and F_c, fragments thereof such as F_{ab} or fragments of the antibodies being idiotypes of peptide epitopes may also be used in an alternative embodiment.

The peptides according to the invention are suitable for preparing an agent for the diagnosis of biologically active hPTH(1-37).

Referring to the following examples, the invention will be described in more detail.

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Example 1

Solid-Phase Synthesis of Peptides

The method of the invention for synthesizing the peptides is based on the peptide synthesis using a solid support. Each of the C-terminal amino acids is bound to the support material in the presence of dicyclohexylcarbodiimide and dimethylaminopyridine. Wang resin or similar resins are used as support material for the syntheses.

The following derivatives of L-amino acids are used in the synthesis of the sequence, starting from the peptidyl resin as specified: a) hPTH(1-10): Fmoc-Asn(Trt)-Wang resin, Fmoc-His(Trt)-OH, Fmoc-Met-OH, Fmoc-Leu-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Val-OH, Boc-Ser(tBu)-OH; b) hPTH(9-18): Fmoc-Met-Wang resin, Fmoc-Ser(tBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Leu-OH, Fmoc-His(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Asn(Trt)-OH, Boc-His(Trt)-OH; c) hPTH(24-37): Fmoc-Leu-Wang resin, Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-Phe-OH, Fmoc-Asn(Trt)-OH, Fmoc-His(Trt)-OH, Fmoc-Val-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Leu-OH.

The synthesis may be carried out by <u>in situ</u> activation using 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) or derivatives thereof, or benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) or derivatives thereof in the presence of diisopropylethylamine or N-methylmorpholine and 1-hydroxybenzotriazole, using a four- to tenfold excess of Fmoc-L-amino acid during the coupling reactions in N,N-dimethylformamide, N,N-dimethylacetamide or N-methylpyrrolidone. Removal of the Fmoc groups is effected using 20% piperidine

or 2% piperidine and 2% 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in N,N-dimethylformamide, N,N-dimethylacetamide or N-methylpyrrolidone. Following synthesis, the resins are washed with 2-propanol and dichloromethane and dried to constant weight in a high vacuum.

Removal from the support and deprotection are carried out by reacting the peptidyl resin with trifluoroacetic acid containing 5% scavenger, water, ethanediol, phenol or thioanisole for 30-90 minutes at room temperature, filtrating, washing with trifluoroacetic acid, and subsequently precipitating with tert-butyl methyl ether. The precipitate is lyophilized from aqueous solution.

Example 2

Purification and Analysis

The raw products are purified by chromatography on a C18 reversed phase column (10 µm, buffer A: 0.01 N HCl in water; buffer B: 20% isopropanol, 30% methanol, 50% water, 0.01 N HCl; gradient: 10-80% within 60 minutes; detection at 230 nm).

The purity of the products is determined using mass spectrometry and C18 reversed phase chromatography.

Example 3

Coupling to Carrier Protein

Used as carrier proteins are hemocyanin, thyroglobulin, bovine serum albumin, ovalbumin, or mouse serum albumin. Coupling is performed according to the carbodiimide method by way of the carboxyl groups of the peptides. The peptide is

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activated in aqueous solution by reaction with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride for 5 minutes. Coupling is effected by adding the activated peptide to an aqueous solution of the carrier. The molar ratio is 1 peptide on 50 amino acids of the carrier protein. The reaction takes 4 hours.

CLAIMS

1. The peptides from hPTH(1-37) having the sequence

hPTH 1-10
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-Asn^{10}-OH (1)

hPTH 1-9
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-OH (2)

hPTH 1-8
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-OH (3)

hPTH 1-7
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-OH (4)

hPTH 1-6
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-OH (5)

hPTH 1-5
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-OH (6)

hPTH 9-18
NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (7)

hPTH 10-18
NH_2-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (8)

hPTH 11-18
NH_2-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (9)

hPTH 12-18
NH_2-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (10)

hPTH 13-18
NH_2-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (11)

hPTH 14-18
NH_2-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (12)

hPTH 9-17

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-OH (13)

hPTH 9-16

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-OH (14)

hPTH 9-15

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-OH (15)

hPTH 9-14

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-OH (16)

hPTH 9-13

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-OH (17)

hPTH 24-37

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-
Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (18)

hPTH 25-37

NH_2-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-
Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (19)

hPTH 26-37

NH_2-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-
Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (20)

hPTH 27-37

NH_2-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-
Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (21)

hPTH 28-37

NH_2-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-
Leu^{37}-OH (22)

hPTH 29-37

NH_2-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (23)

hPTH 30-37

NH_2-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (24)

hPTH 31-37

NH_2-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (25)

hPTH 32-37

NH_2-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (26)

hPTH 33-37

NH_2-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (27)

hPTH 24-36

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-OH (28)

hPTH 24-35

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-OH (29)

hPTH 24-34

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-OH (30)

hPTH 24-33

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-OH (31)

hPTH 24-32

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-OH (32)

hPTH 24-31

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-OH (33)

hPTH 24-29

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-OH (34)

hPTH 24-28

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-OH (35)

2. The peptides according to claim 1, which are modified at the N-terminal end, in the side-chain and/or at the C-terminal end, taking the form of acetylation, amidation, phosphorylation and/or glycosylation products and/or are bound to carrier proteins such as hemocyanin, thyroglobulin, bovine serum albumin, ovalbumin, or mouse serum albumin.

3. A diagnostic agent which can be obtained using the per se known immunization of animals with at least one of the peptides according to claim 1, recovering fractions containing immunoglobulins from the immunized animals, and isolating fractions having an antibody titer against at least one of the peptides according to claim 1, and which optionally contains additional adjuvants and/or vehicles.

4. Antibodies or fragments of antibodies, which can be obtained by immunizing animals with at least one of the peptides according to claim 1.

5. Use of the peptides according to claim 1 for producing an agent for the diagnosis of biologically active hPTH(1-37).

Abstract

The present invention is directed to peptides from the sequence of hPTH(1-37), which contain α-helical amino acid sequence regions and/or non-structured amino acid sequence regions, said peptides being capable of inducing antibodies when injected into animals. Furthermore, the invention is directed to a diagnostic agent and antibodies obtainable by immunizing animals using the peptides according to the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Prof.Dr.Dr. Wolf-Georg Forssmann c/o NIP
(B) STREET: Feodor-Lynen-Str. 31
(C) CITY: Hannover
(D) STATE: Niedersachsen
(E) COUNTRY: Germany
(F) POSTAL CODE: 30625

(ii) TITLE OF INVENTION: Peptide aus der Sequenz des hPTH (1-37)

(iii) NUMBER OF SEQUENCES: 36

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Ser Val Ser Glu Ile Gln Leu Met His Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Ser Val Ser Glu Ile Gln Leu Met His
1 5

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Ser Val Ser Glu Ile Gln Leu Met
1 5

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Ser Val Ser Glu Ile Gln Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Ser Val Ser Glu Ile Gln
1 5

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Ser Val Ser Glu Ile
1 5

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

His Asn Leu Gly Lys His Leu Asn Ser Met
1 5 10

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Asn Leu Gly Lys His Leu Asn Ser Met
1 5

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Leu Gly Lys His Leu Asn Ser Met
1 5

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Gly Lys His Leu Asn Ser Met
1 5

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Lys His Leu Asn Ser Met
1 5

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

His Leu Asn Ser Met
1 5

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

His Asn Leu Gly Lys His Leu Asn Ser
1 5

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

His Asn Leu Gly Lys His Leu Asn
1 5

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

His Asn Leu Gly Lys His Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

His Asn Leu Gly Lys His
1 5

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

His Asn Leu Gly Lys
1 5

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe Val Ala Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Arg Lys Lys Leu Gln Asp Val His Asn Phe Val Ala Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Lys Lys Leu Gln Asp Val His Asn Phe Val Ala Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

Lys Leu Gln Asp Val His Asn Phe Val Ala Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Leu Gln Asp Val His Asn Phe Val Ala Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Gln Asp Val His Asn Phe Val Ala Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Asp Val His Asn Phe Val Ala Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Val His Asn Phe Val Ala Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

His Asn Phe Val Ala Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Asn Phe Val Ala Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe Val Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe Val
1 5 10

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

Leu Arg Lys Lys Leu Gln Asp Val His Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Leu Arg Lys Lys Leu Gln Asp Val His
1 5

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Leu Arg Lys Lys Leu Gln Asp Val
1 5

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

Leu Arg Lys Lys Leu Gln Asp
1 5

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

Leu Arg Lys Lys Leu Gln
1 5

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

Leu Arg Lys Lys Leu
1 5

970208us

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: **KNUT ADERMANN, ET AL.**
App. No.:)
Filed:) Attorney's Docket No. 07826-0007
For: **PEPTIDES FROM THE hPTH (1-37) SEQUENCE**)

VERIFIED STATEMENT (DECLARATION) BY A NON-INVENTOR SUPPORTING A CLAIM BY ANOTHER FOR SMALL ENTITY STATUS

I hereby declare that I am making this verified statement to support a claim by Knut Adermann, Dieter Hock, and Markus Magerlein for small entity status for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, with regard to the invention entitled **PEPTIDES FROM THE hPTH (1-37) SEQUENCE** by inventors named above described in:

(x)the specification filed herewith
( )Application No. _____, filed _____
( )patent No. _____, issued _____

I hereby declare that I would qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying fees under section 41(a) and (b) of Title 35, United States Code, if I had made the above identified invention.

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

(x) no such person, concern, or organization
( ) persons, concerns or organizations listed below*

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

FULL NAME _____

ADDRESS _____
( ) INDIVIDUAL ( ) SMALL BUSINESS CONCERN ( ) NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Prof. Dr. Dr. Wolf-Georg Forssmann

ADDRESS OF PERSON SIGNING: Niedersachsisches Institut für Peptidforschung,
Feodor-Lynen-Str. 31, 30625 Hannover, Germany

DATE: 19.03.97 **SIGNATURE:**

970208 us

DECLARATION AND POWER OF ATTORNEY Attorney's Docket No. **07826-0007**

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name. I believe I am an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: "**PEPTIDES FROM THE hPTH (1-37) SEQUENCE**", the specification of which

☐ is attached hereto.

☒ was filed on September 22, 1995 as PCT International Application No. PCT/EP95/03757 and was amended (if applicable) on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I do not know and do not believe that the same was ever known or used by others in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to the date of this application. I further state that the invention was not in public use or on sale in the United States of America more than one year prior to the date of this application. *I understand that I have a duty of candor and good faith toward the Patent and Trademark Office,* and I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate disclosing subject matter in common with the above-identified specification and having a filing date before that of the application on which priority is claimed:

Country	App. No.	Date of Filing	Priority Claimed Under 35 USC §119
German	DE 44 34 551.8	September 28, 1994	Yes x No

I hereby claim the benefit under Title 35, United States Code, § 120 of any prior United States application(s), or §365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each claim of the present application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations §1.56, which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Application No.	Filing Date	Status: patented, pending, abandoned

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statement were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patents issuing thereon.

I hereby authorize the U.S. attorneys named herein to accept and follow instructions from **Dr. Thomas Godemeyer,** as to any action to be taken in the Patent and Trademark Office regarding this application, without direct communication between the U.S. attorney and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney named herein will be notified by the undersigned.

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POWER OF ATTORNEY: The following attorneys are hereby appointed to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Anthony B. Askew - 24,154; Roger T. Frost - 22,176; Jeffrey E. Young - 28,490; Robert E. Richards - 29,105; John R. Harris - 30,388; Stephen M. Schaetzel -31,416; Larry A. Roberts - 31,871; Thomas A. Hodge - 22,602; Charles L. Warner II - 32,320; Gregory T. Gronholm - 32,415; Dale Lischer - 28,438; Peter G. Pappas - 33,205; James Dean Johnson - 31,771; Nora M. Tocups - 35,712; W. Scott Petty - 35,645; Daniel J. Warren - 34,272; Larry W. Shults - 34,025; Hubert J. Bernhardt III - 36,738; Virginia L. Carron - 37,110; Leona G. Young - 37,266; Jamie L. Greene-32,467; William A. Hartselle - 36,548; Holmes J. Hawkins III - 38,918; Mary Anthony Merchant - 39,771; Michael J. Mehrman - 40,086; William L. Warren - 36,714; Felipe J. Farley - 38,445; F. Leslie Besseger III - 39,108; James A. Witherspoon - 36,723; Brenda M. Ozaki - 40,339; James D. Withers - 40,376.

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Inventor's signature K. Adermann	Date: 12.03.1997

☒ Additional inventors are being named on separately numbered sheets attached hereto.

Attorney Docket No.: 07826-0007
Title: PEPTIDES FROM THE hPTH (1-37) SEQUENCE
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Inventor's signature Markus Mägerlein	Date: 19.03.97